

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C09D 167/04, B05D 7/00, A23C 19/16, C12P 7/62	A1	(11) International Publication Number: WO 96/00263 (43) International Publication Date: 4 January 1996 (04.01.96)
(21) International Application Number: PCT/NL95/00222 (22) International Filing Date: 23 June 1995 (23.06.95) (30) Priority Data: 9401037 23 June 1994 (23.06.94) NL (71) Applicant (for all designated States except US): STICHTING ONDERZOEK EN ONTWIKKELING NOORD NEDERLAND (SOONN) [NL/NL]; Pallasweg 1, P.O. Box 225, NL-8901 BA Leeuwarden (NL). (72) Inventors; and (75) Inventors/Applicants (for US only): EGGINK, Gerrit [NL/NL]; Langenhorst 9, NL-6714 LG Ede (NL). NORTHOLT, Martin, Dinant [NL/NL]; Kerkbuurt 59, NL-9265 LR Suawoude (NL). (74) Agent: DE BRUIJN, Leendert, C.; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report.</i>
(54) Title: METHOD FOR PRODUCING A BIOLOGICALLY DEGRADABLE POLYHYDROXYALKANOATE COATING WITH THE AID OF AN AQUEOUS DISPERSION OF POLYHYDROXYALKANOATE		
(57) Abstract A method for producing a biologically degradable polyhydroxyalkanoate coating in the form of an elastomeric film, wherein an aqueous dispersion of polyhydroxyalkanoate or a mixture of polyhydroxyalkanoates is prepared and the dispersion is applied to the surface to be coated, after which water is made or allowed to evaporate to obtain a polyhydroxyalkanoate film, the film formation taking place at a temperature lower than the melting point of the polyhydroxyalkanoate, such a temperature even for example being more than 35 °C below the melting point and in which the polyhydroxyalkanoate used is a polymer which is made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6-14 and/or is a Pseudomonas polyhydroxyalkanoate other than a polymer or copolymer of β -hydroxyvalerate or β -hydroxybutyrate, without requiring additional steps to render the film elastomeric. Product coated with such a polyhydroxyalkanoate film, in particular a coated product to be applied to or consumed by a human being or animal or a product which must not be exposed to a temperature equal to or more than 100 °C, such as cheese. Elastomeric, preferably water-resistant film of polyhydroxyalkanoate, wherein the polyhydroxyalkanoate is made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6-14 and/or is a Pseudomonas polyhydroxyalkanoate other than a polymer or copolymer of β -hydroxyvalerate or β -hydroxybutyrate.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

METHOD FOR PRODUCING A BIOLOGICALLY DEGRADABLE POLYHYDROXYALKANOATE COATING WITH THE AID OF AN AQUEOUS DISPERSION OF POLYHYDROXYALKANOATE

DESCRIPTION

5 The present invention relates to a method for producing a biologically degradable polyhydroxyalkanoate coating, wherein an aqueous dispersion of polyhydroxyalkanoate is applied to the surface to be coated. In particular, the invention relates to the coating of surfaces which form part of a product to be supplied for consumption to a human being or animal and/or surfaces which form part of a product which is not resistant to high temperatures. A suitable example of a product to be coated is a foodstuff, in particular cheese. The invention also relates to objects coated with such a polyhydroxyalkanoate coating, and also to elastomeric, films of polyhydroxyalkanoate and latices thereof. 10 Elastomeric means the polyhydroxyalkanoates behave at room temperature as elastomers, wherein the crystals serve as physical crosslinks providing a minimum elongation at break of 50%.

Poly(3-hydroxyalkanoates) (PHA) are biologically degradable polymers which can be accumulated by microorganisms as sources of carbon and energy (Anderson and Dawes 1990, Microbiol. Rev. 54:450-472). Poly(3-hydroxybutyrate) (PHB) and the copolymer poly(3-hydroxybutyrate)-co-valerate (PHB/HV) are the most known and best studied forms of PHA and are classified as polyhydroxyalkanoates of the PHB type. In the past ten years, a large number of new types of PHA have been found. In particular, Pseudomonas strains have proved to be capable of synthesizing PHAs with diverse compositions (Lageveen et al. 1987, Appl. Environ. Microbiol. 54, 2924 - 2932). These polyhydroxyalkanoates comprise 3-hydroxy acids having a carbon-chain length of $C_6 - C_{14}$ and are termed polyhydroxyalkanoates of the PHA type. 20 25

30 In order to be able to use polyhydroxyalkanoates, they must be derived from biomass and separated from contaminating cell parts such as proteins and lipids. Two important working-up strategies have been developed for PHB and PHB/HV. The first method relates to an extraction with a solvent such as methylene chloride, dichloroethane, chloroform or trichlorobenzene (Lafferty et al. 1988, Biotechnology vol. 6b, 136 - 176). The disadvantage of this method is that the organic solvents used are toxic and environmentally unacceptable. The use of these solvents would not only make the working-up of PHAs expensive but also considerably limit the number of possible applications of PHAs. All the 35

applications in which PHAs come into contact with products to be applied to or consumed by a human being or animal, such as foodstuffs, are ruled out in this case because small residues of the solvent which is harmful to health can, after all, always be present.

5 In the second working-up method, the bacteria cells containing PHB/HV are broken open by means of a combined physical, chemical and enzymatic procedure. In this way, the PHB/HV granules are released from the biomass and an aqueous dispersion is produced, viz. a latex. The aqueous PHB/HV dispersion is further purified of cell constituents by
10 means of rinsing steps and centrifugation. A dry PHB/HV powder which can be processed further to produce, for example, foils, small bottles, fibres etc. can then be obtained by means of spray drying the dispersion (Holmes 1985, Phys. Technol. 16, 32 - 36).

 With a view to the environmental requirements, great need has
15 arisen for biologically degradable products. This certainly applies to products which comprise a solid support with a coating. Processing of PHA to form such a coating is an interesting field of application. It is known of PHB and the copolymer PHB/HV that they can be processed in various ways to form a coating. In the first place, by applying a PHB
20 solution in an organic solvent such as chloroform to the surface of the coated agent and allowing the solvent to evaporate. It is clear that this is a very unacceptable method for environmental reasons.

 In a second approach, use is made of an aqueous dispersion of PHB, PHB remaining behind as a powder on the surface of the agent to be
25 coated after evaporation of the water. The PHB granules do not fuse and films can only be formed by exposing the surface coated with PHB powder to a solvent such as chloroform (Marchessault et al. 1990, in Novel Biodegradable Microbial Polymers) or by heating the surface coated with PHB powder to the melting point of PHB. This process is disclosed in
30 international patent application WO91/13207 of Marchessault and Lepoutre. Marchessault and Lepoutre describe a method of preparing a coated or impregnated paper or other fibre construct, a latex for use in the preparation of paper or other fibre constructs and films and self supporting films. They describe a latex comprising a colloidal suspension
35 in water of essentially non-crystalline particles of a poly- β -hydroxyalkanoate polymer or copolymer and illustrate butyrate and valerate as preferred hydroxyalkanoates. Their latex will comprise 10 to 50%, preferably 15 to 30%, more preferably 20 to 25% by weight of the PHA. Typically their latex will comprise 10 to 15 parts by weight. They

describe that a film can be made from a latex by casting a dilute solution of 15-25% w/w solids on an impervious surface and allowing the water to evaporate slowly at room temperature. They state that heating the air dried film in a drying oven at a temperature 30 degrees below T_m causes moderate fusion. These films have a microporous structure and can be hot pressed at 100-140°C and 1000-5000 psi to produce flexible translucent films of average to high crystallinity. The hot fusion treating of PHB/V copolymer containing 21% by weight PHV provides water imperviousness. Dense transparent films are formed by exposing air dried films to solvent or liquid vapour atmospheres such as chloroform and other halogenated solvents. Explicitly Marchessault and Lepoutre describe "A few grams of 21% HV polymer latex was diluted to 20-30% w/w solids with distilled water. The resulting mixture was poured on a clean glass surface and allowed to air dry at room temperature overnight. A white uniform coat or film with little or no strength was obtained. It was then placed in a convection oven set at 100°C for a period of not less than 10 min. A partially fused film was obtained". Hot pressing the resulting film provided a film with at most elongation at break of 30%. Alternatively subjecting the partially fused film to chloroform provided an extremely smooth and tough film.

Disadvantages also apply to these known methods, firstly the use of an organic solvent and secondly, the performance of a heating step in which a temperature above 100°C has to be used. The applications are therefore also clearly limited in this case. Marchessault and Lepoutre's main application is for impregnating paper. They disclose nothing with regard to preparing elastomeric films. Their coatings are either microporous and non-peelable or thermoplastic exhibiting at most elongation at break of 30%. They do not disclose elastomeric films suitable for appliance to or consumption by humans or animals.

DE-4 040 158 describes how the use of organic solvents is harmful to health and how the presence of the solvent in the coating makes it impossible to use them in the foodstuffs or pharmaceutical industry, in particular as packing material also for material for treating wounds. A further disadvantage mentioned is that the known coatings are available solely as impermeable films. For many applications, however, such as medical bandaging or other materials for absorbing body fluids, a porous coating, in particular a coating permeable to gas and air, is necessary, which materials cannot be obtained with the aid of the coatings with solvents. In DE 4 040 158 a

dispersion of polyhydroxybutyrate or polyhydroxyvalerate, or copolymers thereof, in water is described for obtaining such a porous coating. The aqueous dispersions can be prepared by stirring PHA powders or directly in the processing of PHA-forming organisms. The coated objects are prepared, according to DE 4 040 158, by applying the dispersion of PHA to the object and then subsequently drying to remove the dispersing agent. Depending on the drying conditions, a more or less porous coating or an impermeable and continuous coating film can be obtained. The degree of porosity can be controlled by the choice of drying conditions and the granule size of the polymer particle. If a continuous and impermeable coating film is desired, heating is carried out to temperatures at which the coating melts to form a film either during the drying or subsequently to the drying. According to DE 4 040 158, a porous non-film-forming coating is advantageous where gas and air permeability is necessary or desirable, as in the case of medicinal bandaging for wound treatment. A porous coating is obtained by not heating the PHA coating to the melting point so that a film is not formed. In certain cases, sintering in the region of the melting point is advantageous to produce coatings having a defined porosity. The melting point is 173.3°C for polyhydroxybutyrate and it is 126.6°C for polyhydroxybutyrate-co-hydroxyvalerate, with a ratio of 80:20 mol%. As examples of surfaces to be coated, mention is made of nonwoven fabric, woven fabric or plastic foil, cellulose, wood, textile fibres, metal or glass.

Various products, in particular food products such as cheese, are coated with a synthetic, non-biologically degradable plastic. The plastic of this rind cannot be recovered or be recycled and presents a problem for the environment. Furthermore, such a coating must fulfil a large number of functions and a large number of requirements. The coatings which have been described above do not fulfil the requirements which are imposed on a cheese coating or a coating for a temperature-sensitive agent. The coating material serves to prevent mechanical damage and also attack by moulds. The coating must offer both mechanical and hygienic protection. Furthermore, the coating must preferably be easy to apply, for example by immersion or coating with a sponge or brush. In particular, in the case of cheese, the coating advantageously affects the lustre and rind formation and it must adhere well to the wet surface of the cheese. In addition, after drying, a coating must be produced which does not crack and does not stick, for example, to boards or other cheeses. The coating should also be semipermeable since CO₂ and

certain other flavouring components must be able to diffuse through the coating in order not to adversely affect maturing. Water vapour must also be allowed to pass through in a controlled manner so that natural maturing can occur without any question of excessive weight loss. On the other hand, components must not diffuse into the cheese from the coating. Furthermore, the coating must keep well during prolonged storage, there must be good adhesion to any paraffin layer to be applied and there must be no peeling after vacuum packaging. In addition, the machines which are used must be easy to clean. It will be clear that these properties apply not only to cheese coatings but are generally desirable.

Although biologically degradable and compostable coatings exist, they are generally water-soluble polymers which are consequently also water-sensitive. Examples of these are polyvinyl alcohol, polyethylene oxide, and also proteins such as gelatine. Hitherto, there has been no possible use of polyhydroxyalkanoate films as a coating in the foodstuffs industry, on the one hand, because of the use of organic solvents and, on the other hand, because of the need to heat the polyhydroxyalkanoates to the melting point, which could unnecessarily damage the product.

The object of the present invention is to solve the problems explained above. The present invention relates therefore to a method for producing a biologically degradable poly(3-hydroxyalkanoate) coating in the form of an elastomeric film, in which an aqueous dispersion of poly(3-hydroxyalkanoate) is prepared and the dispersion is applied to the surface to be coated, after which water is made or allowed to evaporate to obtain a film, the film formation taking place at a temperature lower than the melting point of the polyhydroxyalkanoate, and the polyhydroxyalkanoate used being a polymer which is made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6 - 14. The 3-hydroxy fatty acids can therefore contain 6, 7, 8, 9, 10, 11, 12, 13 or 14 carbon atoms. suitably the method can even be carried out at a temperature more than 35° C below the melting point.

A method for producing a biologically degradable polyhydroxyalkanoate coating in the form of an elastomeric film, in which an aqueous dispersion of polyhydroxyalkanoate is prepared and the dispersion is applied to the surface to be coated, after which the water is made or allowed to evaporate to obtain a film, the film formation taking place at a temperature lower than the melting point of the polyhydroxyalkanoate with a polymer which occurs in *Pseudomonas* being

used as polyhydroxyalkanoate, with the proviso the polyhydroxyalkanoate is not a polymer or copolymer of β -hydroxybutyrate or β -hydroxyvalerate, also forms part of the invention. The method for producing coating in the form of an elastomeric film according to the invention does not require
5 further steps of hot fusion, hot pressing or contact with chloroform or halogenated solvent to obtain the elastomeric characteristic. Selection of specific hydroxyalkanoates as starting material in an aqueous dispersion ensures elastomeric properties are acquired merely upon drying the aqueous dispersion after applying it to the surface to be coated. On
10 the basis of what was known regarding film formation and coatings of polyhydroxybutyrate and polyhydroxyvalerate and copolymers thereof it was totally unexpected that films having an elastomeric character could so simply be obtained. The films according to the state of the art with PHB and PHV required additional measures as described in detail above.
15 Furthermore it could not be predicted that hydroxyalkanoates made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon chain length of 6-14 would be suitable for preparing an aqueous dispersion even having characteristics suitable for use as a coating at all. Such an aqueous dispersion could be expected to have a chewing gum like structure
20 in water or hydrophilic media. It also was not to be expected what the properties of any resulting film would be and certainly not that they would be so fine or so different from those of the described PHB and PHV films. Suitably the method according to the invention can be carried out at a temperature more than 35°C lower than the melting point of the
25 polyhydroxyalkanoate. Preferably, a polymer is used which occurs in fluorescent *Pseudomonas* belonging to the RNA homology group I. The polymers of PHA occurring in fluorescent *Pseudomonas* belonging to the RNA homology group I do not, after all, comprise PHB or PHB/HV and have such low melting points, glass transition temperatures and crystallinity that
30 the film formation takes place at a temperature lower than the melting point of the polyhydroxyalkanoates and can even occur at a temperature more than 35° C below the melting temperature.

PHB and the copolymer PHB/HV, the polyhydroxyalkanoates of the PHB type, are typically thermoplastics having a high melting point (120 -
35 180°C) (Byrom 1987, TIBTECH 5, 246 - 250). These polymers are highly crystalline. PHAs formed by *Pseudomonas*, in particular by fluorescent *Pseudomonas* belonging to the RNA homology group I have, on the other hand, typically elastomeric properties. The melting point of this category of polyhydroxyalkanoates varies between 40 and 60°C and the

crystallinity depends on the composition, but is between 0 and 30% (Marchessault et al. 1990, Ind. J. Biol. Macromol. 12, 158 - 165). Pseudomonas strains can easily be brought in a known manner to high production levels of PHA (Eggink et al. 1992, Proceedings of ACS Division of Polymeric Material 67, 130 - 131). Pseudomonas produces no PHB, and therefore, the polyhydroxyalkanoates which are formed by Pseudomonas can be used in an outstanding way in a method according to the present invention without first extracting PHB therefrom. In particular, because the working up of polyhydroxyalkanoates from microorganisms provides an aqueous dispersion, mixtures of polyhydroxyalkanoates occurring in microorganisms, in particular, are extremely suitable for use in a method according to the present invention. The working-up and coating can be performed continuously and in a minimum number of steps. As already stated, the PHA-containing bacteria cells can be broken open by means of a combined physical, chemical and enzymatic procedure, as a result of which the PHA granules are released from the biomass and an aqueous dispersion is produced which can be purified further from contaminating constituents by means of washing steps and centrifugation. With the present method, spray drying of the dispersion and adding of organic solvents are no longer necessary. The processing of PHA to form a coating can be (carried out) just by applying the dispersion as it is obtainable directly by working-up from the microorganisms and by allowing it to dry. The polyhydroxyalkanoates of the PHB type probably do not form an impermeable film because of the high crystallinity and the high glass transition temperature of 0 - 4°C. the invention in all embodiments (method of preparation, film, coated product, latices) is preferably directed at polyhydroxyalkanoates having a glass transition temperature below 0° C.

In the method according to the invention, a high-temperature step is unnecessary for sintering or melting for the purpose of film formation. in contrast to the method which was known for poly(3-hydroxybutyrate) or poly(3-hydroxybutyrate)-co-valerate coatings. Neither the presence of an organic solvent nor an intermediate step in which the aqueous dispersion of PHA is dried to form a powder and then dissolved in an organic solvent is necessary. With the method according to the present invention, it is therefore possible to coat products which may not be exposed to an excessively high temperature and/or to coat products which are applied to or consumed by a human being or animal and in which organic solvents must not be present. The coating does not per se need,

however, to be applied to or consumed by the animal or the human being.

The method according to the present invention can be performed at a temperature in the range of 0 - 30°C, preferably at a temperature in the range of 10 - 18°C. The choice of temperature employed will depend on the product to be coated and on economic considerations. The PHA or PHAs used will also be a factor on which the temperature to be used depends.

The method according to the invention can be performed with good results using a dispersion whose dry-matter content of PHA is at least 10%. Outstanding results are achieved with a dry-matter content higher than 30% and even higher than 50%. Dispersions with a dry matter content higher than 65% have been shown to be suitable embodiments for use in preparing elastomeric films according to the invention.

The method in which an aqueous dispersion according to the present invention for coating a product is used has been performed by the inventors with PHAs of the PHB type such as PHB/HV and PHB but these did not yield a film, only a powdered coating whose powder granules exhibited no or little mutual adhesion. The known coating could not be peeled off as a film from the coated product. The coating did not have the mechanical strength of a film. The inventors results agree with what has already been stated in DE 4 040 158 and PCT 91/13207 for PHB and the copolymer of PHB with hydroxyvalerate. A film having good mechanical strength and containing PHB granules can only be formed by exposing the surface coated with PHB powder or aqueous dispersion to a solvent such as chloroform or by heating to the melting point.

Polyhydroxyalkanoates of the PHA type with saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length between C₆ and C₁₄ are, inter alia, produced by Pseudomonas strains, in particular fluorescent Pseudomonas strains belonging to the RNA homology group I. This category of hydroxy fatty acids has typically elastomeric properties, with a melting point between 40 and 60°C and a crystallinity between 0 and 30% (Marchessault et al. 1990, Ind. J. Biol. Macromol. 12, 158 - 165) and yields outstanding films by the present method. Preferably, the method according to the invention is performed with polyhydroxyalkanoate copolymers which comprise at least 3-hydroxy acids having carbon-chain lengths of C₈ - C₁₀. Outstanding results are obtained if the mixture comprises at least 50% C₈ - C₁₀ PHAs.

An advantage of the use of dispersions is that standard additives such as stabilizers, plasticizers, fillers, dyes, emulsifiers,

thickening agents, antioxidants, antistatics, preservatives, crosslinking agents, etc. can be added to the dispersion and the properties of the film can be simply improved and modified thereby. In addition, the presence of the water phase offers the possibility of mixing the PHA with
5 other water-soluble, biologically degradable polymers. Other biologically degradable polymers which can be taken up in the dispersion comprise polyvinyl alcohol, gelatine, methylcellulose, polyethene oxide, pvp, starch. Furthermore, an anti-fungal agent can be taken up in the dispersion.

10 As stated above, the method according to the present invention can advantageously be used to coat foodstuffs, in particular for coating cheese. The film which is obtained by the present method is
15 outstandingly suitable for coating cheese. All the requirements which are imposed thereon with respect to a suitable cheese coating are fulfilled by the coating which can be obtained by the present invention. In particular, the mechanical strength of the coating is a positive factor. The fact that the PHA granules yield, without heating to the
20 melting point, a film having sufficient mechanical strength to function as cheese coating is very surprising since PHAs of the PHB type yielded only a powdered coating. The film obtained resembles a "latex" film and is easy to distinguish from a coating which is obtained with an aqueous dispersion of the PHB type without the heating step to the melting point of the PHA. In an embodiment which is particularly suitable for
25 producing a coating or film according to the invention, a drying time of less than 24 hours at an atmospheric humidity of 85% and a temperature of 10 - 18°C may be adequate. This method according to the invention is outstandingly suitable for producing a cheese coating.

The bacteria cells of *Pseudomonas* can readily be cultured, the polyhydroxyalkanoates formed depending on the medium on which the
30 *Pseudomonas* is cultured. Thus, various fatty acids, such as oleic acid and linoleic acid, can be used as hydroxy fatty acids for the PHA to be formed.

Polyhydroxyalkanoates of other microorganisms may, of course, also be used in the method according to the present invention, provided
35 they are saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6 - 14. The composition of the polyhydroxyalkanoates may be controlled by choosing the media on which the microorganisms are cultured so that, on the one hand, the presence of PHB and PHB/HV can be avoided in the polyhydroxyalkanoate mixture to be used for the method

and, on the other hand, the composition of the polyhydroxyalkanoates produced by a microorganism and, consequently, also the properties of the film can be precisely controlled.

The present invention also relates to a product coated with a polyhydroxyalkanoate film, said film being elastomeric and said film comprising polyhydroxyalkanoate made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6 - 14. The invention also comprises a product coated with a polyhydroxyalkanoate film, said film being elastomeric and said film comprising polyhydroxyalkanoate which occurs in *Pseudomonas*, preferably polyhydroxyalkanoate which occurs in fluorescent *Pseudomonas* belonging to the RNA homology group I with the proviso the polyhydroxyalkanoate is not a polymer or copolymer of β -hydroxybutyrate or β -hydroxyvalerate. In particular a coated product according to the invention can be a product to be applied to or consumed by a human being or animal or a product which must not be exposed to 100°C or more. A suitable product is cheese. The product coated with polyhydroxyalkanoate film may also be composed of a mixture of polyhydroxyalkanoates of the abovementioned type. The invention relates to all products coated with polyhydroxyalkanoate film of the above type which can be prepared by the method according to the invention. The products coated with a polyhydroxyalkanoate according to the invention do not consist of poly(3-hydroxybutyrate) or a copolymer thereof, poly(3-hydroxybutyrate)-co-valerate or poly(3-hydroxyvalerate) since, to prepare such coatings, a heating step is necessary to form a film. Besides this these polymers or copolymers do not provide elastomeric films as is clear from the literature cited above.

The invention furthermore comprises an elastomeric film of polyhydroxyalkanoate in which the polyhydroxyalkanoate is made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6 - 14. The invention also comprises an elastomeric film of polyhydroxyalkanoate in which the polyhydroxyalkanoate occurs in *Pseudomonas*, preferably in fluorescent *Pseudomonas* belonging to the RNA homology group I with the proviso the polyhydroxyalkanoate is not a polymer or copolymer of β -hydroxybutyrate or β -hydroxyvalerate. Preferably a film according to the invention is water resistant. In particular, the invention relates to an elastomeric film of polyhydroxyalkanoate which is suitable as a coating for products suitable to be applied to or consumed by human beings and/or animals. The film according to the invention can suitably be applied to hydrophilic

surfaces and will exhibit good attachment properties to such a surface without requiring a heating step or any other step than drying at a temperature below T_g . In fact temperatures more than 35°C below T_g can even be applied. Cheese is a suitable product. A preferred film according to the invention will comprise an elongation at break of at least 50%. Suitably the elongation at break will be over 200%. The dry-matter content of a film according to the invention can be more than 10%. Preferably it is higher than 30%, even higher than 50%. Another property of a film which is suitable in particular as cheese coating is a film which contains less than 4.5% of parts which are soluble in water. The typical gas permeability is 3-5 l/m²/hr at a temperature of 20°C and relative humidity of 90-0% for a film according to the invention. These values being for a film with a thickness of 0.1 mm. Not only is the invention directed at the novel and inventive films but also at latices useful for production of such films as can be produced in the various embodiments of the preparation method of coated products according to the invention.

Thus an aqueous latex-like dispersion of polyhydroxyalkanoate made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon chain length of 6-14 falls within the scope of the invention. Also an aqueous latex-like dispersion of polyhydroxyalkanoate, wherein the polyhydroxyalkanoate occurs in *Pseudomonas*, preferably polyhydroxyalkanoate which occurs in fluorescent *Pseudomonas* belonging to the RNA homology group I, with the proviso the polyhydroxyalkanoate is not a polymer or copolymer of β -hydroxybutyrate or β -hydroxyvalerate falls within the scope of protection. Such dispersions preferably having a pH higher than 8, preferably higher than 10. Suitably the pH is 12 as illustrated in the Examples. A dispersion according to the invention may further comprise more than 30%, preferably more than 50%, in particular more than 65% polyhydroxyalkanoate as dry weight. Preferably an aqueous latex-like dispersion according to any of the aforementioned embodiments is substantially free of compounds toxic to a human being or animal. In addition it is substantially free of contaminant cell rests of cells from which the polyhydroxyalkanoate has been derived.

Use of an aqueous latex-like dispersion according to the invention in any of the embodiments described above for preparing a film or product coated with a film comprising drying the aqueous dispersion at a temperature below the T_g and even more than 35 °C below the T_g of the polyhydroxyalkanoate for obtaining an elastomeric film also falls within

the scope of the invention.

EXAMPLE 1

Preparation of a PHA dispersion

5

The starting point is formed by *Pseudomonas* cells cultured on oleic acid and containing between 30 and 90% PHA. The composition of the polymer is 4.4% 3-hydroxyhexanoate, 33.5% 3-hydroxyoctanoate, 32.2% 3-hydroxydecanoate, 14.4% 3-hydroxydodecanoate and 15.5% cis-5,3,3-hydroxytetradecanoate. A suspension is prepared from the biomass which has a dry-matter content of 10% (weight/volume). 1 mg of lysozyme is added per gram of biomass to break up the peptidoglycan layer. Then 0.1 ml of neutrase (NOVO Nordisk, 0.5 AU/g) is added per gram of cells and incubation is carried out for 60 min at 37°C. After that, 0.1 ml of alcalase (NOVO Nordisk) is added per gram of cells and incubation is carried out for 90 min at 55°C while stirring. Finally, 5 M NaOH solution is added until the pH is 12 and incubation is carried out for 15 min at room temperature. The latex thus produced is washed and concentrated by centrifugation or microfiltration. Scanning electron microscopy reveals that the PHA dispersion is composed of granules which vary in size between 0.4 μm and 2 μm . The PHA dispersion is found to be 90 to 99% pure with the aid of gas-chromatographic analysis and elemental analysis. It has been ascertained that in general a pH higher than 8 during working up of the PHA biomass to the latex dispersion improves the working up efficiency.

EXAMPLE 2

Preparation of a PHA coating on cheese

30

The PHA dispersion described in Example 1 can be applied to a cheese surface by brushing or immersion. For a dry-matter content of 20 to 25%, the drying time is approximately 24 hours at a temperature of 12°C and an atmospheric humidity of 85%. After drying, a dry, smooth, water-resistant film with good adhesion is produced. The elongation at break is approximately 400%. The typical gas permeability is 3-5 $\text{l/m}^2/\text{hr}$ at a temperature of 20°C and relative humidity of 90-0%. These values being for a film with a thickness of 0.1 mm. This is comparable with that of PHA which is purified with the aid of chloroform.

35

EXAMPLE 3

Blending PHA with water-soluble, biologically degradable polymers

The abovementioned water-soluble and biologically degradable
5 polymers can readily be mixed with the PHA when the latter is still in
the latex phase. The starting point is formed by the latex mentioned in
Example 1, to which said polymers can be added as a solution or as a
powder, the powder form preferably taking place in quantities which can
10 dissolve in the aqueous phase of the latex. Thus, PHA and water-soluble
polymer can be mixed with one another in any desired ratio. The polymers
soluble in the aqueous phase have a favourable effect on the stability
and the homogeneity of the latex. The properties of the coating as
formed from such a latex can differ appreciably with respect to the pure
15 PHA coating, especially as regards the mechanical and adhesion
properties, the gas permeability and the water sensitivity. The
differences which can be achieved thereby are within the field of a
person skilled in the art and fall within the scope of protection of the
present invention.

20 EXAMPLE 4

Crosslinking of PHA during or after drying the latex

Depending on the composition of the substrate, the PHA may
contain unsaturated carbon bonds in the side groups. Under the influence
25 of, for example, UV radiation, sulphur or peroxides, these may mutually
form crosslinks and thus join chains and parts of chains of the polymer
to one another. A number of physical properties of the PHA film formed
can be altered by incorporating crosslinks. Solubility and mechanical
properties, tackiness, and the prevention of the formation of a
30 crystalline fraction can be controlled in this way. The starting point
is formed by a latex which is worked up as in Example 1 with a PHA in
which a quantity of monomer containing unsaturated (carbon) bonds is
incorporated. Drying produces a film from the latex which then loses its
thermoplastic nature under the influence of light, air and/or additions
35 of certain substances to the latex as a result of the formation of
crosslinks. The material thereby loses its melting point. The latex can
also be modified or blended in the ways mentioned above in Examples 2 and
3.

CLAIMS

1. Method for producing a biologically degradable polyhydroxyalkanoate coating in the form of an elastomeric film, wherein an aqueous dispersion of polyhydroxyalkanoate or a mixture of
5 polyhydroxyalkanoates is prepared and the dispersion is applied to the surface to be coated, after which water is made or allowed to evaporate to obtain a polyhydroxyalkanoate film, wherein the film formation takes place at a temperature below the melting point of the polyhydroxyalkanoate, such a temperature for example being more than 35°C
10 lower than the melting point and wherein the polyhydroxyalkanoate used is a polymer which is made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6 - 14, said method not requiring further steps of hot fusion, hot pressing or contact with chloroform or halogenated solvents to obtain the elastomeric characteristic.
- 15 2. Method for producing a biologically degradable polyhydroxyalkanoate coating in the form of an elastomeric film, wherein an aqueous dispersion of polyhydroxyalkanoate or a mixture of polyhydroxyalkanoates is prepared and the dispersion is applied to the surface to be coated, after which water is made or allowed to evaporate
20 to obtain a polyhydroxyalkanoate film, wherein the film formation takes place at a temperature lower than the melting point of the polyhydroxyalkanoate and such a temperature for example being more than 35°C lower than the melting point and wherein the polyhydroxyalkanoate used is a polymer which occurs in Pseudomonas, preferably a polymer which
25 occurs in fluorescent Pseudomonas belonging to the RNA homology group I, said method not requiring further steps of hot fusion, hot pressing or contact with chloroform or halogenated solvents to obtain the elastomeric characteristic with the proviso the polyhydroxyalkanoate is not a polymer or copolymer of β -hydroxybutyrate or β -hydroxyvalerate.
- 30 3. Method according to Claim 1, wherein the polyhydroxyalkanoate used is a polymer which occurs in Pseudomonas, preferably a polymer which occurs in fluorescent Pseudomonas belonging to the RNA homology group I.
4. Method according to one of the preceding claims, wherein polyhydroxyalkanoates are used which are made up of at least 50% 3-
35 hydroxy acids having a carbon-chain length of $C_8 - C_{10}$.
5. Method according to one of the preceding claims, wherein the dispersion is applied and the film formation takes place at a temperature in the range of 0 - 30°C, preferably at a temperature in the range of 10 - 18°C.

6. Method according to one of the preceding claims, wherein a dispersion is used of which the dry-matter content is at least 10%, preferably more than 30%, more preferably more than 50%.

7. Method according to one of the preceding claims, wherein, after
5 applying the dispersion, the coated surface is subjected to crosslinking, such as by means of exposure to one or more of UV, peroxides, sulphur and a chemical crosslinker.

8. Method according to one of the preceding claims, wherein the dispersion also comprises another biologically degradable polymer, such
10 as polyvinyl alcohol, gelatine, methylcellulose, polyethene oxide, polyvinylpyrrolidone or starch.

9. Method according to one of the preceding claims, wherein the dispersion also comprises an anti-fungal agent.

10. Method according to one of the preceding claims, wherein the
15 dispersion also furthermore comprises one or more additives from the group comprising dyes, emulsifiers, thickening agents and stabilizers.

11. Method according to one of the preceding claims, wherein the surface to be coated forms part of a product to be applied to or consumed by a human being or animal, such as a foodstuff.

20 12. Method according to one of the preceding claims, wherein a product is coated which must not be exposed to a temperature equal to or above 100°C.

13. Method according to one of the preceding claims, wherein the product to be coated is cheese.

25 14. Product coated with an elastomeric polyhydroxyalkanoate film, wherein the film comprises polyhydroxyalkanoate made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6 - 14.

15. Product coated with an elastomeric polyhydroxyalkanoate film, wherein the film comprises polyhydroxyalkanoate which occurs in
30 Pseudomonas, preferably polyhydroxyalkanoate which occurs in fluorescent Pseudomonas belonging to the RNA homology group I with the proviso the polyhydroxyalkanoate is not a polymer or copolymer of β -hydroxybutyrate or β -hydroxyvalerate.

16. Product according to claim 14 or 15 to be consumed by or to be
35 applied to a human being or animal.

17. Product according to any of claims 14-16 which must not be exposed to a temperature equal to or more than 100°C, such a product for example being cheese.

18. Elastomeric film of polyhydroxyalkanoate, wherein the

polyhydroxyalkanoate is made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon-chain length of 6 - 14.

19. Elastomeric film of polyhydroxyalkanoate, wherein the polyhydroxyalkanoate occurs in *Pseudomonas*, preferably polyhydroxyalkanoate which occurs in fluorescent *Pseudomonas* belonging to the RNA homology group I, with the proviso the polyhydroxyalkanoate is not a polymer or copolymer of β -hydroxybutyrate or β -hydroxyvalerate.

20. An aqueous latex-like dispersion of polyhydroxyalkanoate made up of saturated or unsaturated 3-hydroxy fatty acids having a carbon chain length of 6-14.

21. An aqueous latex-like dispersion of polyhydroxyalkanoate, wherein the polyhydroxyalkanoate occurs in *Pseudomonas*, preferably polyhydroxyalkanoate which occurs in fluorescent *Pseudomonas* belonging to the RNA homology group I, with the proviso the polyhydroxyalkanoate is not a polymer or copolymer of β -hydroxybutyrate or β -hydroxyvalerate.

22. An aqueous latex-like dispersion according to claim 20 or 21 having a pH higher than 8, preferably higher than 10.

23. An aqueous latex-like dispersion of polyhydroxyalkanoate according to any of claims 20-22 comprising more than 30%, preferably more than 50%, in particular more than 65% polyhydroxyalkanoate as dry weight.

24. An aqueous latex-like dispersion according to any of claims 20-23, substantially free of compounds toxic to a human being or animal.

25. An aqueous latex like dispersion according to any of claims 20-24, substantially free of contaminant cell rests of cells from which the polyhydroxyalkanoate has been derived.

26. Use of an aqueous latex-like dispersion according to any of claims 20-25 for preparing a film or product coated with a film comprising drying the aqueous dispersion at a temperature below the T_m and even more than 35 °C below the T_m of the polyhydroxyalkanoate for obtaining an elastomeric film.

INTERNATIONAL SEARCH REPORT

Intern: J Application No
PCT/NL 95/00222

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C09D167/04 B05D7/00 A23C19/16 C12P7/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C09D C08G C12P A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 13207 (PULP AND PAPER RESEARCH) 5 September 1991 see claims 1,10 ---	1,5,14, 15,18-20
X	EP,A,0 274 151 (RIJKSUNIVERSITEIT GRONINGEN) 13 July 1988 see claims 1,2,29 see page 5, line 25 - line 36 ---	14,15, 18,19
A	DE,A,40 40 158 (PCD PETROCHEMIE) 17 June 1992 cited in the application see claims 1,2,4,5 see page 2, line 34 - line 64 ---	1
A	WO,A,92 18553 (KOHAP LTD.) 29 October 1992 see claims 1,2 ---	1
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

16 August 1995

Date of mailing of the international search report

08.09.95

Name and mailing address of the ISA

European Patent Office, P.B. 5318 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Van Puymbroeck, M

INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/NL 95/00222

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 11656 (FIRMENICH SA.) 10 June 1993 see claims 1,7 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 95/00222

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9113207	05-09-91	NONE	
EP-A-0274151	13-07-88	NL-A- 8603073	01-07-88
		CA-A- 1335370	25-04-95
		DE-D- 3789377	21-04-94
		DE-T- 3789377	28-07-94
		JP-A- 63226291	20-09-88
		US-A- 5135859	04-08-92
		US-A- 5334698	02-08-94
DE-A-4040158	17-06-92	NONE	
WO-A-9218553	29-10-92	AU-A- 8612191	17-11-92
		EP-A- 0582567	16-02-94
		JP-T- 7500615	19-01-95
		US-A- 5395919	07-03-95
WO-A-9311656	10-06-93	EP-A- 0569569	18-11-93
		JP-T- 6505403	23-06-94
		US-A- 5422257	06-06-95

